

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/09169 A2

(51) International Patent Classification⁷: C07K 5/062,
5/065, C07D 295/12, A61K 38/05, 31/5375, 31/381, A61P
19/00, 31/00, 35/00, 37/00, 9/00

(21) International Application Number: PCT/GB00/02830

(22) International Filing Date: 21 July 2000 (21.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9917909.5 31 July 1999 (31.07.1999) GB

(71) Applicant (for all designated States except US): NAEJA
PHARMACEUTICAL INC [CA/CA]; #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SINGH, Rajeshwar
[CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA). ZHOU, Nian
[CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA). REDDY,
Andhe, V., N. [CA/CA]; Naeja Pharmaceutical Inc, #2,
4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
THOMAS, George [CA/CA]; Naeja Pharmaceutical Inc,
#2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
DING, Qizhu [CA/CA]; Naeja Pharmaceutical Inc, #2,
4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
KALETA, Jadwiga [CA/CA]; Naeja Pharmaceutical

Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2
(CA). MICETICH, Ronald, George [CA/CA]; Naeja
Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton,
Alberta T6E 5V2 (CA). WHITTAKER, Mark [GB/GB];
British Biotech Pharmaceuticals Ltd., Watlington Road,
Cowley, Oxford OX4 5LY (GB).

(74) Agents: WALSH, David, Patrick et al.; Appleyard Lees,
15 Clare Road, Halifax HX1 2HY (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

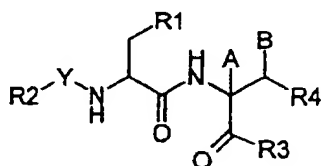
(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: CYSTEINE PROTEASE INHIBITORS



(I)

p-toluenesulfonic acid salts.

(57) Abstract: This invention relates to derivatives
of alpha-amino acid amides, to pharmaceutical
compositions containing such compounds, and to their
use in medicine as inhibitors of cysteine proteases,
particularly the cathepsins. A compound of formula
(I) is described or a pharmaceutically acceptable salt,
hydrate or solvate thereof. Pharmaceutically acceptable
salts of the compounds of this invention include the
sodium, potassium, magnesium, calcium, hydrogen
chloride, tartaric acid, succinic acid, fumaric acid and

WO 01/09169 A2

Cysteine Protease Inhibitors

This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such
5 compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins.

Background to the Invention

The cathepsin family (C1) of lysosomal cysteine (or thiol)
10 proteases is a subset of the papain superfamily (clan CA of cysteine proteases) and includes cathepsin B, H, K, S, L and the recently discovered cathepsins O, O2/K, V, X, Z and W (lymphopain). Related enzymes also regarded as
15 cysteine proteases are the cytoplasmic Ca^{2+} dependent calpains (family C2). Cysteine proteases are classified both functionally and according to the nature of their active site, which has a thiol residue. They differ in substrate specificities and other enzymatic activities, these differences probably arising from evolutionary
20 divergence.

The known cathepsins are synthesized on membrane bound ribosomes, transferred to the endoplasmic reticulum, then to the Golgi apparatus and finally to the lysosome and
25 endosomes. They have an important function in regulation of intracellular protein metabolism, mobilisation of tissue proteins and conversion of proenzymes, prohormones and neuropeptides into biologically active molecules. The cathepsins are believed to be involved in a number of
30 diseases.

Cathepsin K can be secreted into the extracellular space and is involved in bone and cartilage remodelling. Cathepsin K is implicated in the pathogenesis of osteoporosis. Cathepsin K inhibitors can prevent
5 osteoporosis in animal models (PNAS.1997. 94:14249-14254). Cathepsin L inhibitors have also been shown to inhibit osteoporosis (Bone, 1997. 20:465-471).

Cathepsin B and other cysteinyl cathepsins have also been
10 shown to be released extracellularly by various tumour cells and are thought to play a role in tumour invasion (Journal of cellular Physiology. 1992. 150:534-544).

The cysteinyl cathepsins have also been shown to play a
15 role in rheumatoid arthritis (Arthritis and Rheumatism 1994. 37:236-247) and neuronal and cardiac ischaemia (European Journal of Neuroscience. 1998. 10.1723-1733).

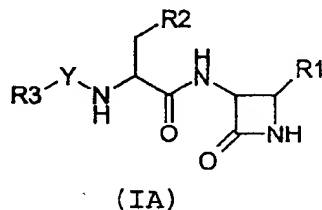
Cathepsins S and L both play a role in the generation of
20 free MHC class II molecules capable of binding antigenic peptides in the endosomes. These class II/peptide complexes move to the cell membrane and are involved in T lymphocyte activation. Inhibitors of Cathepsin S have been shown to inhibit allergic immune responses (Journal of
25 Clinical Investigation. 1998. 101:2351-2363).

In addition to their role in the above diseases, cysteinyl cathepsins play a major role in the pathogenesis of infectious diseases. For example, cysteinyl cathepsins are
30 used by the protozoal parasites Plasmodium (malaria) and Trypanosoma (Chagas Disease) to invade the human host and

cysteinyl cathepsin inhibitors can inhibit experimental disease in both cases (Antimicrobial agents and chemotherapy. 1998. 42:2254-2258; Journal of Experimental Medicine. 1998. 188:725-734). Cysteinyl cathepsins are also virulence factors for several pathogenic bacteria.

A recent review (Annu. Rev. Physiol. 1997. 59:63-88) describes the state of the art of cysteine proteases, including the cathepsins, and their presumed biological functions. Other reviews deal with cathepsin B inhibitors as potential anti-metastatic agents (Exp. Opin. Ther. Patents, 1998, 8: 645-672), and cathepsin K inhibitors as potential treatments for osteoporosis (Exp. Opin. Ther. Patents, 1999, 9: 683-644).

International patent applications WO 96/32408, WO 98/12176, WO 98/12210 and GB 9805287.0 describe, inter alia, classes of cysteine protease inhibitors which may be represented by formula (IA):



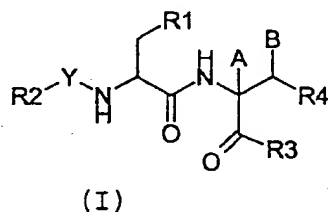
wherein Y, R₁, R₂ and R₃ represent the groups found in corresponding positions of the compounds disclosed in those publications. These known compounds are azetidin-2-ones which are monosubstituted at positions 3 and 4.

Brief Description of the Invention

The present invention makes available a new class of cysteine protease inhibitors which differ in structure from those disclosed in WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moiety, as more fully explained below. These compounds are useful for the treatment of diseases mediated by cysteine protease activity, for example muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, and protozoal or bacterial disease.

15 Detailed Description of the Invention

According to the present invention, there is provided a compound of formula (I)



wherein:

Y represents $-C(O)-$ or $-S(O_2)-$;

25 R₁ represents a radical of formula R₆-(ALK)_p-(Z)_n-(ALK)_q-
wherein Z represents -O- or -S-, ALK represents a
divalent C₁-C₃alkyl or halogen-substituted C₁-C₃alkyl

radical, p and q are independently 0 or 1, n is 0 or 1 when q is 1 and n is 0 when q is 0, and R₆ is hydrogen or an optionally substituted C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group; or R₁ together with the carbon atom to which it is attached forms a cycloalkyl ring;

R₂ represents -OR₅ or -R₅;

10 R₅ represents a radical of formula R₇-(A)_t- wherein t is 0 or 1; A represents (i) an optionally substituted divalent C₁-C₆alkyl, radical which may be interrupted by one or more non-adjacent -O-, -S- or -NH- linkages, or (ii) a divalent C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R₇ represents hydrogen or an optionally substituted C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

20 R₃ represents (i) an optionally substituted C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR₈ or N(R₈)₂ or (iii) OR₈ wherein R₈ represents hydrogen or an optionally substituted C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl or aryl;

A and B taken together represent a bond and R₄ represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R₄ each independently represents a hydroxy or substituted hydroxy group;

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

- 5 Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.
- 10 As used herein the term (C_1-C_6) alkyl or lower alkyl means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl,
- 15 and hexyl. Similar terms such as " (C_1-C_3) alkyl" are to be interpreted similarly.

As used herein the term C_2-C_6 alkenyl" means a straight or branched chain alkenyl moiety having from 2 to 6 carbon

20 atoms having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl. Similar terms such as " (C_2-C_3) alkenyl" are to be interpreted similarly.

25

As used herein the term " C_2-C_6 alkynyl" means a straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple

30 bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butyne, 2-methyl-2-propynyl, 2-pentyne, 3-pentyne, 4-pentyne, 2-hexynyl, 3-hexynyl, 4-

hexynyl and 5-hexynyl. Similar terms such as "(C₂-C₃)alkynyl" are to be interpreted similarly.

As used herein the term cycloalkyl means a saturated alicyclic moiety having from 3-7 carbon atoms and includes, for example, cyclohexyl, cycloheptyl, cyclopentyl, cyclobutyl and cyclopropyl.

As used herein the term "halogen" means fluoro, chloro, bromo or iodo.

As used herein the term "aryl" refers to a mono-, bi- or tri-cyclic, substituted or unsubstituted, carbocyclic aromatic group, and to groups consisting of two covalently linked substituted or unsubstituted monocyclic carbocyclic aromatic groups. Illustrative of such groups are phenyl, biphenyl and naphthyl. Examples include C₆-C₁₂ aryl groups such as phenyl, biphenyl, naphthyl, tetrahydronaphthyl, dihydronaphthyl, and cyclohexyl phenyl.

As used herein the unqualified term heterocyclyl or heterocyclic means a 5-7 membered heterocyclic ring, which may be aromatic or non-aromatic, containing one or more heteroatoms selected from S, N and O, and optionally fused to a benzene or hetero-atom containing ring. The term therefore includes C₁-C₁₁ heterocyclic groups containing 1-4 heteroatoms selected from nitrogen, sulfur or oxygen. Examples include thienyl, pyridyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3,4-tetrazolyl, imidazolyl, quinolinyl, isoquinolinyl, indolyl, pyrimidinyl, benzofuranyl, benzothienyl, morpholinyl,

thiomorpholinyl, piperazinyl, piperidinyl,
tetrahydroquinolinyl, tetrahydroisoquinolinyl,
pyridylphenyl, pyrimidylphenyl, pyrrolyl, furyl, thienyl,
piperidinyl, imidazolyl, oxazolyl, thiazolyl,
5 thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl,
pyrimidinyl, morpholinyl, piperazinyl, indolyl,
benzimidazolyl, maleimido, succinimido, and phthalimido
groups.

10 As used herein, the term "primary or secondary amino
group" means an amino group carrying one or two
substituents respectively, for example selected from amino
protecting groups, (C₁-C₆)alkyl-X-, (C₂-C₆)alkenyl-X-, (C₂-
C₆)alkenyl-X-, aryl(C₁-C₆)alkyl-X-, aryl(C₂-C₆)alkenyl-X-,
15 aryl(C₂-C₆)alkenyl-X-, heterocyclic(C₁-C₆)alkyl-X-,
heterocyclic(C₂-C₆)alkenyl-X-, heterocyclic(C₂-C₆)alkenyl-
X-, wherein -X- represents a bond or a carbonyl -C(O)-,
sulphonyl -S(O₂)-, or oxycarbonyl -O-C(O)- group, and
wherein any of the foregoing may be substituted. The term
20 "secondary amino group" also means a substituted or
unsubstituted cyclic amino group, such as piperidyl
, morpholinyl, thiomorpholinyl, piperazinyl, pyrrolidinyl
or azetidiny.

25 As used herein, the term "substituted hydroxy group" means
a protected hydroxy group or a hydroxy group substituted
by, for example, any of the substituents listed in the
preceding paragraph as substituents of primary or
secondary amino groups except those wherein X is an
30 oxycarbonyl -O-C(O)- group.

As used herein in contexts other than "substituted hydroxy group", the unqualified term "substituted" as applied to a group or radical means substituted with 1, 2, or 3 substituents selected from

5

(C₁-C₃)alkyl;

phenyl;

hydroxy or mercapto;

(C₁-C₃)alkoxy or (C₁-C₃)alkylthio;

10

phenoxy or phenylthio;

halogen;

trifluoromethyl;

nitro;

cyano (-CN);

15

carboxyl, and amidated, esterified or protected carboxyl;

amino, mono- or di-(C₁-C₃)alkylamino, or protected amino;

(C₁-C₃)alkylcarbonyl- or (C₁-C₃)alkylcarbonylamino-;

20

-CONHR^A, -NHR^A, -NR^AR^B, or -CONR^AR^B wherein R^A and R^B are independently (C₁-C₃)alkyl; and

-NH-C(=NR₉)R₁₀ wherein R₁₀ is amino, mono- or di-(C₁-C₆)alkylamino, protected amino, or (C₁-C₃)alkyl, and R₉ is hydrogen, (C₁-C₃)alkyl, or an N-protecting

25

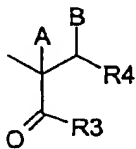
group.

As used herein the term "protecting group" when used in relation to an amino, hydroxy or carboxylic acid moiety in the compounds of this invention means a group which is used to render the amino, hydroxy or carboxylic acid moiety substantially non reactive, ie to neutralise its

30

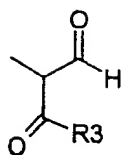
amino, hydroxy or carboxylic acid functionality. In this context, protected amino groups include amido and acylamino, protected hydroxy groups include ethers, protected carboxyl groups include esters, and imidazolyl, indolyl or guanidyl groups may be protected as t-butoxycarbonyl derivatives. These are only examples of the many protecting derivatives known in the art, and others will be known to the skilled man. Such protecting groups are of course well known, eg from the art of peptide synthesis, and are discussed in the widely used handbook by T.W. Greene and P.G.M. Wuts, Protective groups in Organic Synthesis, 2nd Edition, Wiley, New York 1991, and elsewhere in the chemical literature.

As mentioned above, the compounds of the invention differ in structure from those of WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moiety. That substituted carbonylmethyl moiety is the radical (II):



(II)

which may be regarded as notionally derived from the aldehyde radical (III):



(III)

The substituents R_1 and R_2 in the compounds of the invention may be any of the groups falling within the
 5 above definitions of R_1 and R_2 which are present in corresponding positions of cysteine protease inhibitors disclosed in WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0. Without prejudice to the generality of the foregoing, in the compounds of the invention:

10

Y may be, for example, $-C(O)-$;

R_1 may be, for example, a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy,
 15 methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy-phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl; tetrahydroquinolinyl; tetrahydronaphthyl;
 20 aminonaphthyl; or acetamidonaphthyl. Presently preferred are phenyl, isopropyl, cyclohexyl and 3-pyridinyl.

R_2 may be, for example, benzyloxy, 3-phenylpropyloxy, 3-phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-
 25 dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl, methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-

(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-prop-1-enyl.

R₃ may be, for example, methyl, ethyl, isopropyl, t-butyl, cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl, pyridyl, -NH₂, methylamino, dimethylamino, benzylamino, piperidino, morpholino, piperazino, N-methylpiperazino or methoxy, ethoxy, t-butyloxy or phenoxy.

10 When A and B taken together represent a bond, R₄ may be, for example, -NH₂, acetylamino, methylamino, dimethylamino, benzylamino, morpholino, piperidino, morpholino, piperazino or N-methylpiperazino, methoxycarbonylmethylamino, (methoxycarbonyl)-
15 phenethylamino, -OH, methoxy, allyloxy, benzyloxy.

Specific compounds of the invention include those named and characterised in the Examples herein.

20 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-
25 amino-acrylamide
2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-acrylamide
30 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(morpholin-4-yl)-acrylamide

- 2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3- (2-hydroxyethylamino) -acrylamide
2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3-phenylamino-acrylamide
- 5 2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3-piperidino-acrylamide
2E) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3-acetamido-acrylamide
(2Z) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
10 acetamido) -3-acetamido-acrylamide
2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3,3-dihydroxy-propionamide
2- (2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-
acetamido) -3,3-dihydroxy-propionamide
- 15 2- (2S-2-benzyloxycarbonylamino-2-benzyl-acetamido) -3,3-
dihydroxy-propionamide
2- [2S-2- (3-phenylpropionoylamino) -2-benzyl-acetamido] -3,3-
dihydroxy-propionamide
2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
20 acetamido) -3-hydroxy-acrylamide
2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3-benzylamino-N-benzyl-acrylamide
2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3- (4-methylpiperazino) -acrylamide
- 25 2- (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido) -3- (3-tert-butoxycarbonylamino-pyrrolidino) -
acrylamide
(2E) - (2S-2-benzyloxycarbonylamino-2-cyclohexymethyl-
acetamido) -3-acetamido-acrylamide
- 30 (2Z) - (2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-
acetamido) -3-acetamido-acrylamide

- tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate
- 5 Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- 10 Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- 15 2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.
- 2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetophenone.
- 20 2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.
- 2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-fluoroacetophenone.
- 25 (2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide
- (2Z)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide
- 30 (2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide

As stated, the compounds of the invention are inhibitors
5 of cysteine proteases, for example cathepsins B, L, S
and/or K. The invention therefore also provides a
pharmaceutical composition containing a compound of
formula (I) as defined above, and a pharmaceutically
acceptable carrier. Also provided is the use of such a
10 compound in the preparation of a composition for
inhibiting cysteine protease activity in the body of a
mammal suffering a disease mediated by such activity, and
a method of treatment of an animal suffering from a
disease mediated by cysteine protease activity, which
15 method comprises administering to the mammal a sufficient
amount of a compound of formula (I) as defined above to
inhibit such activity.

Diseases mediated by cysteine protease activity include
20 muscular dystrophy, osteoporosis, tumour metastasis,
rheumatoid arthritis, neuronal or cardiac ischaemia,
allergic immune response, and protozoal or bacterial
disease.

25 Compositions with which the invention is concerned may be
prepared for administration by any route consistent with
the pharmacokinetic properties of the active
ingredient(s).

30 Orally administrable compositions may be in the form of
tablets, capsules, powders, granules, lozenges, liquid or

gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

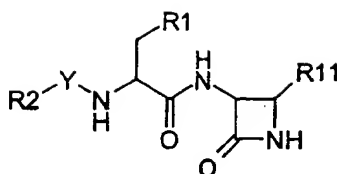
For topical application to the skin, the active ingredient(s) may be made up into a cream, lotion or

ointment. Cream or ointment formulations, which may be used for the drug, are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceuticals such as the British
5 Pharmacopoeia.

The active ingredient(s) may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended
10 or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. Intravenous infusion is another route of administration for the compounds.

15 Safe and effective dosages for different classes of patient and for different disease states will be determined by clinical trial as is required in the art. It will be understood that the specific dose level for any
20 particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the
25 particular disease undergoing therapy.

Compounds of the invention wherein A and B taken together represent a bond and R₄ represents NH₂ may be prepared by treatment of azetidin-2-ones of formula (IV) with ammonium
30 hydroxide.



(IV)

wherein R₁₁ is a leaving group such as phenoxy, acetoxy .

5

Compounds of the invention wherein A and B taken together represent a bond and R₄ represents a primary or secondary amino group may be prepared by treatment of compounds (IV) with a primary or secondary amine, or by appropriate
10 derivatisation of the amino group of the corresponding compounds wherein R₄ is amino.

Compounds of the invention wherein A and B taken together represent a bond and R₄ represents a hydroxy group may be
15 prepared by treatment of compounds (IV) with acetic acid, for example at ambient temperatures. Compounds of the invention wherein A represents hydrogen and B and R₄ represents a hydroxy group may also be prepared by treatment of compounds (IV) with acetic acid, but under
20 less forcing conditions than for the alpha-beta unsaturated compounds, for example at low temperatures such as about 0°C.

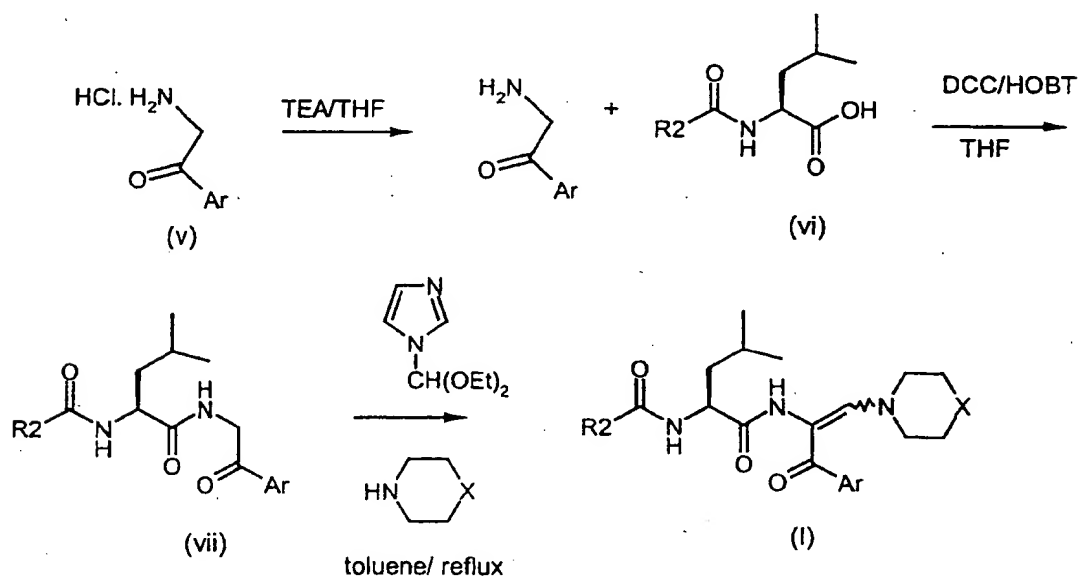
Compounds of the invention wherein A and B taken together
25 represent a bond and R₄ represents a substituted hydroxy group or a primary or secondary amino group may be prepared from the corresponding compounds wherein R₄ is

hydroxy or amino by appropriate derivatisation of that hydroxy or amino group. Likewise, compounds of the invention wherein A represents hydrogen and B and R₄ are independently a substituted hydroxy group may be prepared
 5 from the corresponding compounds wherein B and R₄ are hydroxy by appropriate derivatisation of one or both of those hydroxy groups.

Compounds of the invention wherein A and B taken together
 10 represent a bond and R₄ represents an alkyl, alkenyl, alkynyl, cycloalkyl or aryl may be prepared by the following the synthetic scheme as depicted below in scheme 1.

15

Scheme-1

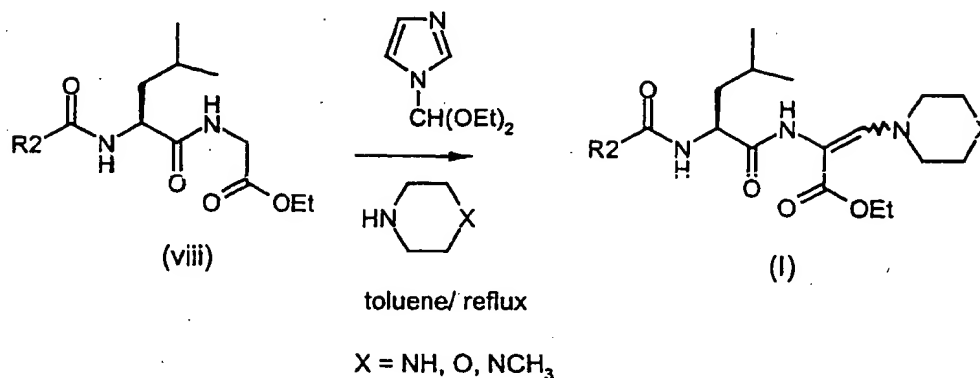


Compounds of the invention wherein A and B taken together represent a bond and R₄ represents an alkoxy, aryloxy or

cycloalkoxy may be prepared by the following the synthetic scheme as depicted below in scheme 2.

5

Scheme-2



In the above processes, the reactants are reacted together with solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Depending on the reactants, a solvent will generally be selected from the group consisting of benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, water, pyridine, acetone and the like. Solvent mixtures may also be utilized.

Reaction temperatures generally range from between -70 °C to 150 °C. The preferred molar ratios of reactants are 1:1 to 5. The reaction time range from 0.5 to 72 hours, depending on the reactants.

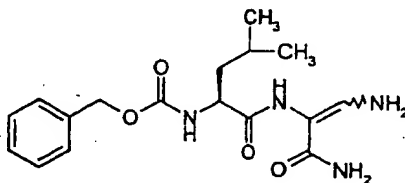
5

The azetidine-2-one strating materials (V) may be prepared by literature methods, including those in International patent applications WO 96/32408, WO 98/12176, WO 98/12210.

10 The following Examples illustrate embodiments of the invention.

Example 1

15 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-
acetamido)-3-amino-acrylamide



A solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (3.0 g, 7.6 mmole) in acetonitrile
20 (50 ml) and 15 ml of ammonium hydroxide (28% NH₃ in water) was stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 1.86 g of the title
25 compound was obtained as white solid.

Yield: 70%

m.p.: 80-90 °C.

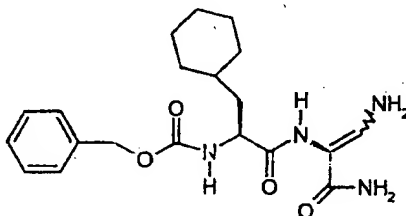
¹H-NMR: (DMSO-d₆), (ppm): 0.7-1.0 (6H, m), 1.4-1.75 (3H, m), 3.9-4.1 (1H, m), 5.02 (2H, s), 5.56 (1.4H, br), 6.26 (2H, s), 6.44 (0.3H, t, J=9 Hz), 6.95 (0.6H, br), 7.12 (0.7H, t, J=9 Hz), 7.25-7.45 (5H, m), 7.36 (0.3H, d, J=6.6 Hz), 7.62 (0.7H, d, J=6.6 Hz), 8.50 (0.7H, s), 8.67 (0.3H, s).

MS (ES⁺): 349 (M+H), calcd for C₁₇H₂₄N₄O₄ 348.

10

Example 2

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide



15

By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-2-one.

20

Yield: 70 %

m.p.: 80-85 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.7-1.95 (13H, m), 3.9-4.15 (1H, m), 5.01 (2H, s), 5.45-5.65 (1.6H, br), 6.26 (2H, s), 6.43 (0.2H, t, J=10 Hz), 6.9-7.0 (0.4H, br), 7.12 (0.8H, t, J=10 Hz), 7.3-7.45 (5H, m), 7.58 (0.2H, d,

25

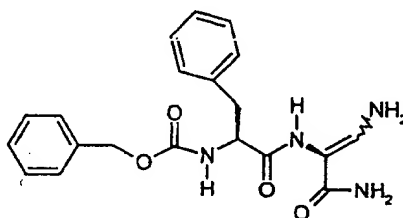
J=6.4 Hz), 7.62 (0.8H, d, J=6.4 Hz), 8.51 (0.8H, s), 8.66 (0.2H, s).

MS (ES+): 389 (M+H), calcd for C₂₀H₂₈N₄O₄ 388.

5

Example 3

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-acrylamide



10 By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-4-acetoxyazetidin-2-one.

Yield: 71 %

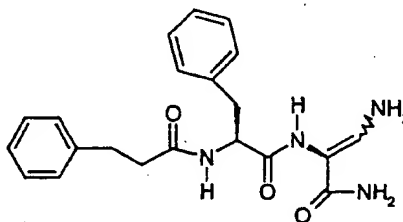
15 m.p.: 127-135 °C.

¹H-NMR: (DMSO-d₆), (ppm): 2.8-3.2 (2H, m), 4.2-4.4 (1H, m), 4.97 (2H, s), 5.3-5.55 (2H, br), 6.10 (2H, s), 7.14 (1H, t, J=11 Hz), 7.2-7.4 (10H, m), 7.78 (1H, d, J=6.4 Hz), 8.6 (1H, s).

20 MS (ES+): 383 (M+H), calcd for C₂₀H₂₂N₄O₄ 382.

25

Example 4

2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-amino-acrylamide

5 By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-4-acetoxyazetidin-2-one.

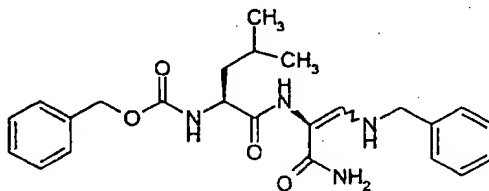
Yield: 75 %

10 m.p.: 75-80 °C.

¹H-NMR: (DMSO-d₆), (ppm): 2.35-2.45 (2H, m), 2.70-3.15 (4H, m), 4.25-4.50 (1H, m), 5.25-5.50 (2H, br), 6.08 (2H, s), 7.06 (1H, t, J=11 Hz), 7.2-7.4 (10H, m), 8.32 (1H, d, J=6 Hz), 8.45 (1H, s).

15 MS (ES⁺): 381 (M+H), calcd for C₂₁H₂₄N₄O₃ 380.

Example 5

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-acrylamide

To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in acetonitrile (5 ml) and water (1 ml), benzylamine (542 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 200 mg of the title compound was obtained as white solid.

Yield: 90%

m.p.: 115-120 °C.

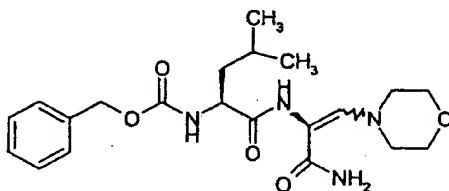
¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.8 (9H, m), 3.9-4.1 (1H, m), 4.29 (2H, d, J=5.5 Hz), 4.9-5.1 (2H, m), 5.9-6.1 (0.7H, m), 6.28 (2H, s), 6.61 (0.3H, d, J=12 Hz), 7.17 (0.7H, d, J=12 Hz), 7.25-7.45 (10H, m), 7.60 (0.3H, d, J=6.5 Hz), 7.66 (0.7H, d, J=6.5 Hz), 8.35-8.55 (0.3H, m), 8.60 (0.7H, s), 8.72 (0.3H, s).

MS (ES⁺): 439 (M+H), calcd for C₂₄H₃₀N₄O₄ 438.

20

Example 6

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(morpholin-4-yl)-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in

acetonitrile (5 ml) and water (1 ml), morpholine (444 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the solid was washed with ether. 200 mg of the title compound was obtained as white solid.

Yield: 90%

m.p.: 120-130 °C.

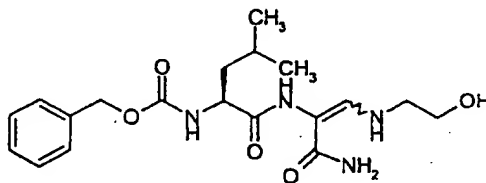
¹H-NMR: (DMSO-d₆), (ppm): 0.8-0.95 (6H, m), 1.3-1.75 (3H, m), 2.6-2.75 (4H, m), 3.5-3.6 (4H, m), 3.9-4.1 (1H, m), 5.00 (2H, s), 6.32 (2H, s), 7.07 (1H, s), 7.36 (5H, m), 7.67 (1H, d, J=6.7 Hz), 8.8 (1H, s).

MS (ES⁺): 419 (M+H), calcd for C₂₁H₃₀N₄O₅ 418.

15

Example 7

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(2-hydroxyethylamino)-acrylamide



20 To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml), hydroethylamine (32 mg, 0.53 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel

25

column chromatography using methanol-chloroform as eluant.

70 mg of the title compound was obtained as white solid.

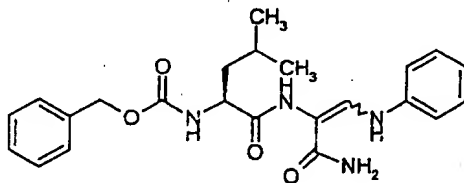
Yield: 70%

m.p.: 89-92 °C.

- 5 ¹H-NMR: (DMSO-d₆), (ppm): 0.8-0.95 (6H, m), 1.4-1.75 (3H, m), 3.05-3.2 (2H, m), 3.35-3.5 (2H, m), 3.95-4.1 (1H, m), 4.65 (1H, t, J=5 Hz), 5.02 (2H, s), 5.4-5.55 (1H, m), 6.75 (2H, s), 7.12 (1H, d, J=12 Hz), 7.35 (5H, m), 7.65 (1H, d, J=6 Hz), 8.55 (1H, s).
- 10 MS (ES+): 393 (M+H), calcd for C₁₉H₂₈N₄O₅ 392.

Example 8

- 15 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-phenylamino-acrylamide



- Aniline hydrochloride (500 mg, 3.8 mmole) was neutralised with Na₂CO₃ solution (600 mg, 5.7 mmole) and
- 20 then extracted with ethyl acetate. After removal of solvent, aniline was dissolved in acetonitrile and added to a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml).
- 25 The resulting mixture was stirred at room temperature overnight. After removal of solvent under vacuum and

lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.
Yield: 10%

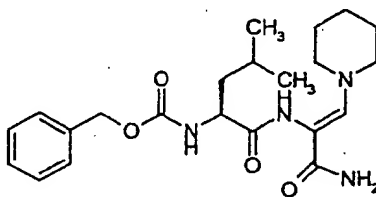
5 m.p.: 199-200 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.85-1.05 (6H, m), 1.5-1.8 (3H, m), 4.0-4.15 (1H, m), 5.09 (2H, s), 6.73 (2H, s), 6.88 (1H, t, J=7.2 Hz), 7.05 (2H, d, J=8 Hz), 7.24 (2H, d, J=7.5 Hz), 7.35 (5H, m), 7.7-7.9 (3H, m), 8.89 (1H, s).

10 MS (ES+): 425 (M+H), calcd for C₂₃H₂₈N₄O₄ 424.

Example 9

15 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-piperidino-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), piperidine (88 mg, 1.06 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant.
25 70 mg of the title compound was obtained as white solid.

Yield: 70%

m.p.: 99-103 °C.

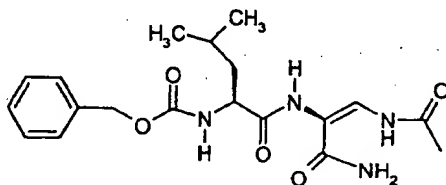
¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.7 (15H, m), 3.2-3.3 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.23 (2H, s), 7.08 (1H, s), 7.3-7.4 (5H, m), 7.63 (1H, d, J=6 Hz), 8.76 (1H, s).

MS (ES⁺): 417 (M+H), calcd for C₂₂H₃₂N₄O₄ 416.

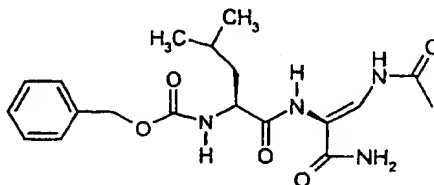
10

Example 10a and 10b

(2E) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido) -3-acetamido-acrylamide (10a)



15 (2Z) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido) -3-acetamido-acrylamide (10b)



150 mg (0.43 mmole) of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (from example 1) was dissolved in acetic anhydride (5 ml) and stirred at room temperature for 2 days. After removal of acetic anhydride, the residue was

purified by silica gel column chromatography using methanol-chloroform as eluant. 40 mg of the title compound (10a) and 45 mg of the title compound (10b) were obtained as white solid.

5 For (10a):

Yield: 24 %

m.p.: 73-76 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.0 (6H, m), 1.45-1.75 (3H, m), 2.08 (3H, s), 3.95-4.1 (1H, m), 5.02 (2H, s),
10 6.90 (1H, br), 7.08 (1H, d, J=12 Hz), 7.3-7.4 (5H, m), 7.5 (1H, br), 7.70 (1H, d, J=6 Hz), 9.23 (1H, s), 10.98 (1H, d, J=12 Hz).

MS (ES+): 391 (M+H), calcd for C₁₉H₂₆N₄O₅ 390.

For (10b):

15 Yield: 27 %

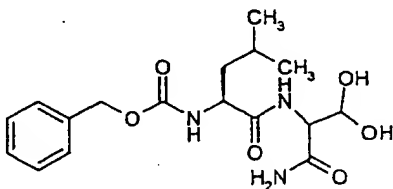
m.p.: 120-123 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.0 (6H, m), 1.5-1.8 (3H, m), 2.01 (3H, s), 4.0-4.1 (1H, m), 5.06 (2H, s),
7.02 (2H, s), 7.3-7.45 (5H, m), 7.64 (1H, d, J=11.4 Hz),
20 7.84 (1H, d, J=6.2 Hz), 9.00 (1H, s), 9.13 (1H, d, J=11.4 Hz),

MS (ES+): 391 (M+H), calcd for C₁₉H₂₆N₄O₅ 390.

Example 11

25 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3,3-dihydroxy-propionamide



To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (30 mg, 0.086 mmole) (from example 1) in acetonitrile (2 ml) and water (0.5 ml), 5 drops of formic acid was added at 0 °C. The mixture was stirred at 0 °C for 1 hr. After removal of acetonitrile under vacuum, precipitate was formed by addition of water (2 ml). The solid was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.

Yield: 32%

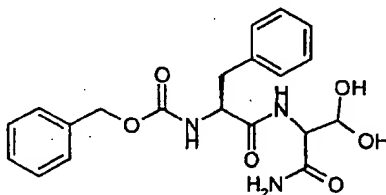
m.p.: 86-90 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.0 (6H, m), 1.4-1.75 (3H, m), 4.0-4.3 (2H, m), 4.6-4.8 (1H, m), 5.02 (1H, s), 5.03 (1H, s), 6.35-6.45 (1H, m), 6.6-6.7 (1H, m), 7.2 (2H, br), 7.3-7.4 (5H, m), 7.5-7.9 (2H, m).

MS (ES+): 350 (M-H₂O+H), calcd for C₁₇H₂₅N₃O₆ 367.

Example 12

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3,3-dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide (from example 2).

Yield: 45%

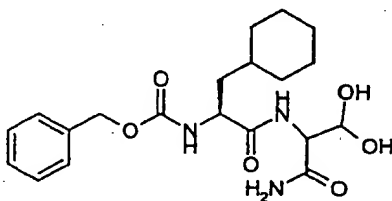
m.p.: 105-110 °C.

¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.8 (13H, m), 4.0-4.3
(2H, m), 4.6-4.85 (1H, m), 5.03 (2H, m), 6.3-6.7 (2H,
5 m), 7.14 (2H, br), 7.3-7.4 (5H, m), 7.45-7.9 (2H, m).

MS (ES+): 390 (M-H₂O+H), calcd for C₂₀H₂₉N₃O₆ 407.

Example 13

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3,3-
10 dihydroxy-propionamide



By a similar method as described in example 11, the
title compound was obtained from 2-(2S-2-
15 benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-
acrylamide (from example 3).

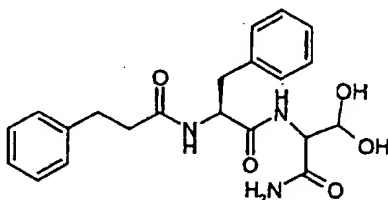
Yield: 40%

m.p.: 98-103 °C.

¹H-NMR: (DMSO-d₆), (ppm): 2.7-3.15 (2H, m), 4.2-4.5
20 (2H, m), 4.65-4.8 (1H, m), 4.94 (2H, s), 6.35-6.5 (1H,
m), 6.6-6.75 (1H, m), 7.1-7.45 (12H, m), 7.5-7.65 (1H,
m), 7.9-8.15 (1H, m).

MS (ES+): 384 (M-H₂O+H), calcd for C₂₀H₂₃N₃O₆ 401.

Example 14

2-[2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido]-3,3-dihydroxy-propionamide

5

By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido)-3-amino-acrylamide (from example 4).

10 Yield: 48%

m.p.: 105-110 °C.

¹H-NMR: (DMSO-d₆), (ppm): 2.3-2.4 (2H, m), 2.6-3.2 (4H, m), 4.2-4.3 (1H, m), 4.5-4.8 (2H, m), 6.3-6.45 (1H, m), 6.55-6.7 (1H, m), 7.1-7.45 (12H, m), 7.85-8.35 (2H, m).

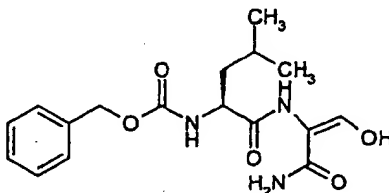
15

MS (ES⁺): 382 (M-H₂O+H), calcd for C₂₁H₂₅N₃O₅ 399.

20

25

Example 15

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-hydroxy-acrylamide

5

To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (55 mg, 0.158 mmole) (from example 1) in acetonitrile (3 ml) and water (0.5 ml), 10 drops of formic acid was added at 0 °C. The mixture was stirred at room temperature for 1 hr. After removal of solvent under vacuum, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 20 mg of the title compound was obtained as white solid.

15 Yield: 36%

m.p.: 105-115 °C.

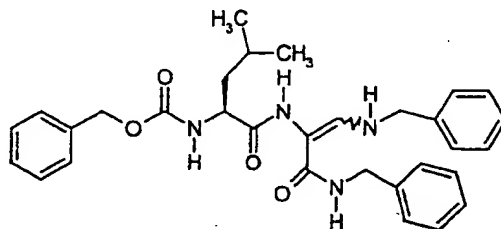
¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.0 (6H, m), 1.4-1.8 (3H, m), 3.9-4.15 (1H, m), 5.02 (2H, s), 6.5-7.1 (1.5H, m), 7.3-7.5 (7H, m), 7.65-7.8 (1H, m), 9.05 (1H, s), 10.2 (0.5H, m).

20

MS (ES⁺): 350 (M+H), calcd for C₁₇H₂₃N₃O₅ 349.

25

Example 16

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-N-benzyl-acrylamide

5 A solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-phenoxy-azetidin-2-one (1.1 g, 3.3 mmole) in ethanol (20 ml) and 5 ml of benzylamine was stirred at room temperature overnight. After removal of solvent under
10 vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 0.96 g of the title compound was obtained as white solid.

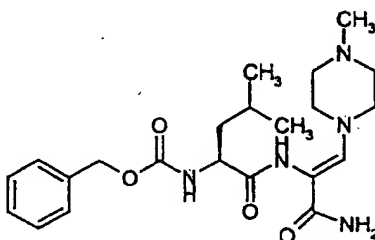
Yield: 55%

15 $^1\text{H-NMR}$: ($\text{DMSO}-d_6$), (ppm): 0.8-1.0 (6H, m), 1.4-1.8 (3H, m), 3.9-4.1 (1H, m), 4.2-4.4 (4H, m), 4.7-4.9 (2H, m), 6.0-6.2 (0.5H, m), 6.6-6.8 (1H, m), 7.1-7.4 (16H, m), 7.6-7.8 (1H, m), 8.3-8.5 (0.5H, m), 8.68 (0.5H, s), 8.85 (0.5H, s).

20 MS (ES⁺): 529 (M+H), calcd for $\text{C}_{31}\text{H}_{36}\text{N}_4\text{O}_4$ 528.

Example 17

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(4-methylpiperazino)-acrylamide



5 To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), 4-methylpiperazine (106 mg, 1.06 mmole) was added and stirred at room
10 temperature overnight. After removal of acetonitrile under vacuum, the residue was dissolved in ethyl acetate and washed with water, brine and dried with Na₂SO₄. After removal of solvent, 30 mg of the title compound was obtained as white solid.

15 Yield: 27%

m.p.: 93.5-95 °C.

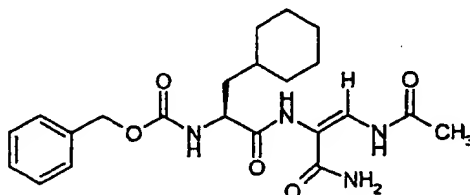
¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.0 (6H, m), 1.3-1.8 (3H, m), 2.13 (3H, s), 2.2-2.35 (4H, m), 3.2-3.35 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.29 (2H, s), 7.07
20 (1H, s), 7.3-7.4 (5H, m), 7.66 (1H, d, J=6.7 Hz), 8.79 (1H, s).

MS (ES⁺): 432 (M+H), calcd for C₂₂H₃₃N₅O₄ 431.

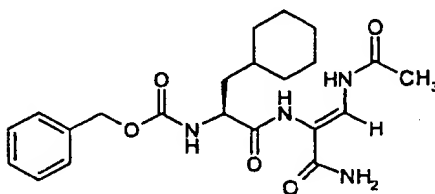
MS (ES+): 518 (M+H), calcd for $C_{26}H_{39}N_5O_6$ 517.

Example 19a and 19b

(2E) - (2S-2-benzyloxycarbonylamino-2-cyclohexymethyl-
5 acetamido) - 3-acetamido-acrylamide (19a)



(2Z) - (2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-
acetamido) - 3-acetamido-acrylamide (19b)



10 By a similar method as described in example 10, the
title compound was obtained from 2-(2S-2-
benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-
amino-acrylamide (example 2).

For (19a):

15 Yield: 21 %

m.p.: 140-142 °C.

$^1\text{H-NMR}$: (DMSO- d_6), (ppm): 0.7-1.8 (13H, m), 2.01 (3H,
s), 3.95-4.1 (1H, m), 5.02 (2H, m), 6.88 (1H, br),
7.07 (1H, d, $J=11$ Hz), 7.3-7.4 (5H, m), 7.5 (1H, br),
20 7.65 (1H, d, $J=6$ Hz), 9.20 (1H, s), 10.97 (1H, d, $J=11$
Hz).

MS (ES+): (M+H), calcd for $C_{22}H_{30}N_4O_5$ 430.

For (19b):

Yield: 41 %

m.p.: 151-153 °C.

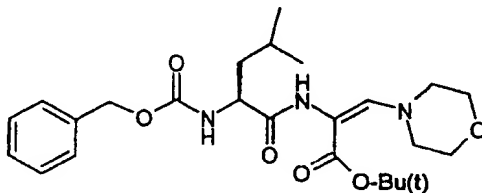
¹H-NMR: (DMSO-d₆), (ppm): 0.8-1.8 (13H, m), 1.99 (3H, s), 4.0-4.1 (1H, m), 5.04 (2H, m), 7.01 (2H, br), 7.3-7.4 (5H, m), 7.62 (1H, d, J=11.4 Hz), 7.81 (1H, d, J=6.2 Hz), 8.98 (1H, s), 9.11 (1H, d, J=11.4 Hz),

MS (ES+): (M+H), calcd for C₂₂H₃₀N₄O₅ 430.

10

Example 20

tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (20)



15 A mixture of ZLeuGlyOBu^t (0.5g, 0. mmol), 1-(diethoxymethyl)imidazole (0.36g, mmol) and camphorsulphonic acid (0.056g, mmol) in toluene was treated with morpholine (1.0ml, mmol) and was refluxed for 24hrs. Solvent was removed in vacuo and the crude product
20 obtained was purified over silica gel column chromatography using a gradient mixture of hexane and ethyl acetate (1:1 to 2:1) gave 20 mg of title compound.

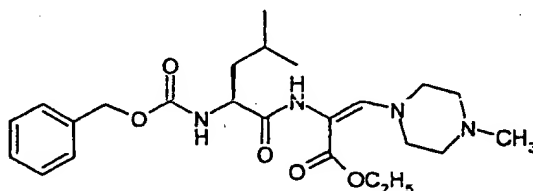
Yield; 3.2%

¹H NMR (DMSO- d₆): δ 0.80-0.90(m, 6H), 1.24-1.72(m, 3H),
25 3.2-3.48(m, 8H), 3.95-4.06(m, 1H), 5.00(AB_q, 2H, J= 2.7

and 13.0Hz), 7.11(s, 1H), 7.34(s, 5H), 7.43(d, 1H, J=8.0Hz), 8.49(s, 1H).

Example 21

5 Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-
acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate
 (21)

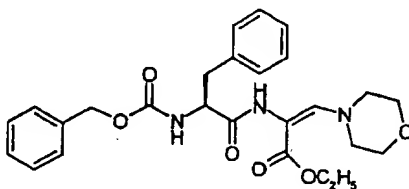


A mixture of ZLeuGlyOEt (0.3g, 0.86mmol), 1-(diethoxymethyl)imidazole (0.28g, 1.27mmol) in toluene was treated with N-methylpiperazine (0.94ml, 8.5mmol) and was refluxed for 24hrs. Solvent was removed in vacuo and the crude product obtained was purified over. Purification of the above crude product over silica gel column chromatography using a mixture of ethyl acetate and methanol (9:1) gave the title compound (0.025g), Yield 6.3%, m. p. 184°C.

¹H NMR (DMSO- d₆): δ 0.87-0.95 (m, 6H), 1.12 (t, 3H, J= 7.0Hz), 1.43-1.79 (m, 3H), 2.11 (s, 3H), 2.21 (s, 4H), 3.40 (m, 4H), 3.94 (q, 2H, J= 7.0Hz), 3.97-4.12 (m, 1H), 5.01 (AB_q, 2H, J= 8.3 and 12.6Hz), 7.20 (s, 1H), 7.34 (s, 5H), 7.44 (d, 1H, J= 7.9Hz), 8.53 (s, 1H).

Example 22

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (22)



5 By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole. Yield; 4.5%,

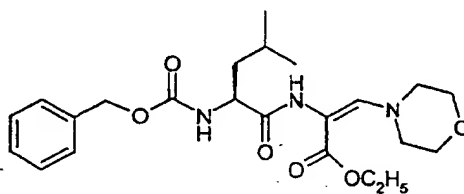
10 m.p. 215-217 °C

¹H NMR (DMSO-d₆): δ 1.14(t, 3H, J= 7.1Hz), 2.69-3.10(m, 2H), 3.35-3.47(m, 8H), 3.98(q, 2H, J= 6.2Hz), 4.20-4.33(m, 1H), 4.93(AB_q, 2H, J= 6.7 and 12.7Hz), 7.20-7.40(m, 11H), 7.57(d, 1H, J= 8.4Hz), 8.79(s, 1H).

15

Example 23

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (23)



20 By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-

(benzyloxycarbonylamino)-2- isopropylmethyl-acetamido]-
acetate, morpholine and 1-(diethoxymethyl)imidazole

Yield; 16%,

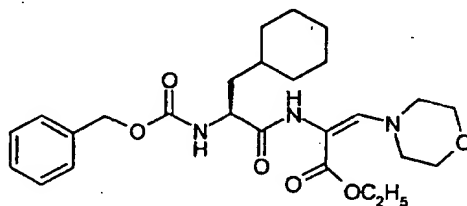
m.p. 139-141 °C

- 5 ¹H NMR (DMSO-d₆): δ 0.87(t, 6H, J= 6.1Hz), 1.13(t, 3H, J= 7.1Hz), 1.43-1.78(m, 3H), 3.38-3.51(m, 8H), 3.90-4.09(m, 3H), 5.00(AB_q, 2H, J= 2.1 and 12.7Hz), 7.21(s, 1H), 7.34(s, 5H), 7.45(d, 1H, J= 7.5Hz), 8.56(s, 1H).

10

Example 24

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-
acetamido]-2-(morpholino-1-methylenyl)-acetate (24)



15

By a similar method as described in example 21, the
title compound compound was obtained from Ethyl-2-[2S-
(benzyloxycarbonylamino)-2- cyclohexylmethyl-acetamido]-
acetate, morpholine and 1-(diethoxymethyl)imidazole.

20 Yield; 10%,

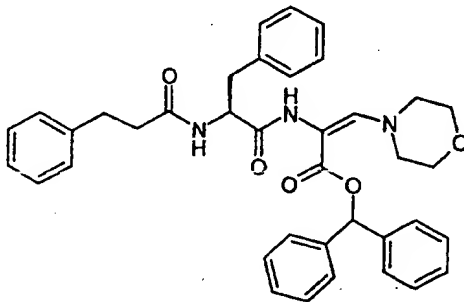
m.p. 202°C

¹H NMR (DMSO-d₆): δ 0.75-1.76(m, 16H), 3.35-3.50(m, 8H),
3.90-4.12(m, 3H), 5.00(s, 2H), 7.21(s, 1H), 7.34(s, 5H),
7.45(d, 1H, J= 7.0Hz), 8.54(s, 1H).

25

Example 25

Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-
phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-
 5 acetate



By a similar method as described in example 21, the
 title compound compound was obtained from Diphenylmethyl-
 10 2-[2S-(benzyloxycarbonylamino)-2- isopropylmethyl-
 acetamido]-acetate, morpholine and 1-
 (diethoxymethyl)imidazole.

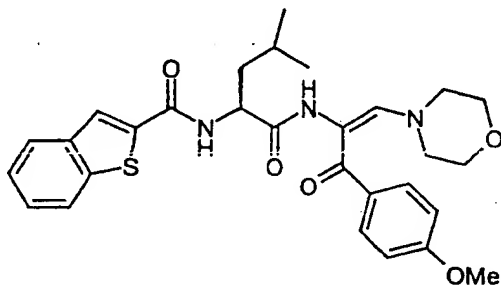
Yield; 27%,

m.p. 167-170 °C

15 ¹H NMR (DMSO-d₆): 2.32 (t, 2H, J= 8.4Hz), 2.67 (t, 2H, J= 7.0Hz), 2.70-3.26 (m, 2H), -3.40-3.55 (m, 8H), 4.50-4.67 (m, 1H), 6.74 (s, 1H), 7.10-7.49 (m, 20H), 8.27 (d, 1H, J= 8.0Hz), 8.95 (brs, 1H).

Example 26

2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-
acetamido]-2-(morpholino-1-methylenyl)-4'-
5 methoxyacetophenone (26).



A mixture of N-(benzothiophene-2-carbonyl)amino-Leucine (0.332g, 1.14mmol), DCC(0.235g, 1.14mmol) and 1-
10 hydroxy benzotriazole (0.154g, 1.14mmol) in dry THF was stirred under nitrogen at r.t. for 1h and cooled to 0 °C. The suspension obtained was filtered and to the filtrate was added 2-amino-4'-methoxy acetophenone (0.23g, 1.14mmol) followed by triethyl amine (0.127g, 1.25mmol).
15 The reaction mixture was stirred at r.t. for 6 hrs. and evaporated in vacuo to give the crude product. Purification of the above crude product over silica gel column chromatography using a mixture of hexane and ethyl acetate (2:3) gave 288 mg of title compound 2[2S-
20 (benzothiophene-2-carbonyl)amino-2-isopropylmethyl-acetamido]-(4'-methoxy)acetophenone.

Yield; 58%,

¹H NMR (DMSO- d₆): δ 0.90-1.00(m, 6H), 1.58-1.82(m, 3H), 3.84(s, 3H), 4.55-4.67(m, 3H), 7.05(d, 2H, J=8.8Hz), 7.44-

7.48(m, 2H), 7.95-8.05(m, 4H), 8.28(s, 1H), 8.35(t, 1H, J=5.9Hz), 8.86(d, 1H, J=8.3Hz).

A mixture of 2-[2S-(benzothiophene-2-carbonyl)-amino-2-isopropylmethyl-acetamido]-4'-methoxyacetophenone

5 (0.271g, 0.618mmol) and 1-(diethoxymethyl)imidazole (0.202g, 0.927mmol) in toluene was treated morpholine (0.269g, 3.09mmol) and refluxed at 130 °C over 22 hrs. Toluene was removed in vacuo and the crude product was purified over silica gel column chromatography using a
10 mixture of ethyl acetate and methanol (9:1) to give 200 mg of title compound.

Yield : 60%.

m.p. 134-136 °C

¹H NMR (DMSO- d₆): δ0.82-0.93(m, 6H), 1.15-1.87(m, 3H),
15 3.40-3.56(m, 8H), 3.75(s, 3H), 4.37-4.50(m, 1H), 6.90(d, 2H, J=8.6Hz), 7.09(s, 1H), 7.41-7.47(m, 4H), 7.95-8.05(m, 2H), 8.25(s, 1H), 8.81(d, 1H, J=7.7Hz), 8.98(s, 1H).

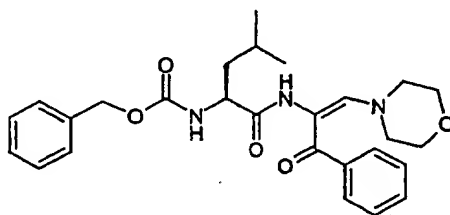
20

25

30

Example 27

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetophenone (27).



5 By following the procedure as described in example 25, the title compound was obtained from 2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-acetophenone, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 53%,

10 m.p. 107-109 °C

¹H NMR (DMSO-d₆): δ 0.78-0.85(m, 6H), 1.06-1.63(m, 3H), 3.40-3.55(m, 8H), 3.90-4.05(m, 1H), 5.00(AB_q, 2H, J= 2.0 and 10.7Hz), 7.13(s, 1H), 7.35(s, 5H), 7.38s, 5H), 7.44(d, 1H, J= 8.0Hz), 8.81(s, 1H).

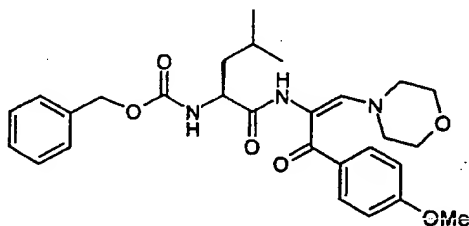
15

20

25

Example 28

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-
acetamido]-2-(morpholino-1-methylenyl)-4'-
methoxyacetophenone (28).



5

By following the procedure as described in example 25,
the title compound was obtained from 2-[2S-
(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-4'-
10 methoxyacetophenone, morpholine and 1-
(diethoxymethyl)imidazole.

Yield; 53%,

m.p. 140-143 °C

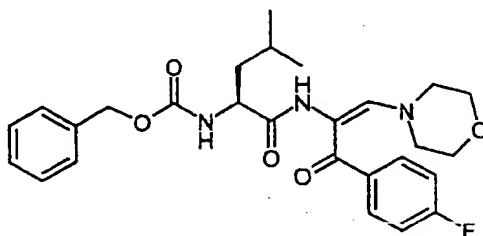
¹H NMR (DMSO-d₆): δ 0.77-0.85(m, 6H), 1.17-1.63(m, 3H),
15 3.40-3.53(m, 8H), 3.75(s, 3H), 3.90-4.05(m, 1H), 4.99(s
2H), 6.89(d, 2H, J= 8.6Hz), 7.07(s, 1H), 7.33-7.45(s, 8H),
8.80(s, 1H).

20

25

Example 29

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-
acetamido]-2-(morpholino-1-methylenyl)-4'-
fluoroacetophenone (29).



5

By following the procedure as described in example 25, the title compound was obtained from 2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-4'-fluoroacetophenone, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 30%,

m.p. 156-157 °C

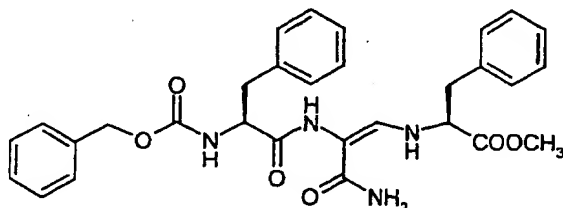
¹H NMR (DMSO-d₆): δ 0.80-0.90 (m, 6H), 1.05-1.64 (m, 3H),
15 3.50 (m, 8H), 3.87-4.03 (m, 1H), 5.00 (s, 2H), 7.15-7.50 (m, 11H), 8.85 (s, 1H).

20

25

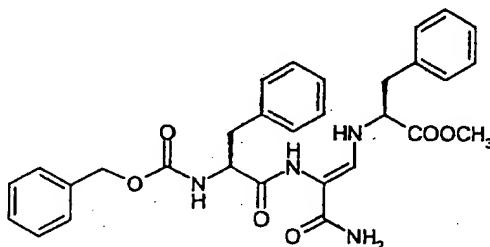
Example 30a and 30b

(2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-
acetamido) -3- (1-carbomethoxy-2-phenethylamino) -acrylamide
(30a)



5

(2Z) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-
acetamido) -3- (1-carbomethoxy-2-phenethylamino) -acrylamide
(30b)



10

By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of phenylalanine.

15 For 30a:

Yield; 34%,

m.p. 71-73 °C

NMR(DMSO-d₆): 2.75-3.03(m, 4H), 3.63(s, 3H), 4.12-4.38(m, 2H), 4.96(s, 2H), 6.00 and 6.55(2br. S, 2H), 6.34(d, 1H,

J= 12.2Hz), 7.20-7.46(m, 15H), 7.67(d, 1H, J= 6.0Hz), 8.30-8.40(m, 1H), 8.70(s, 1H).

For 30b:

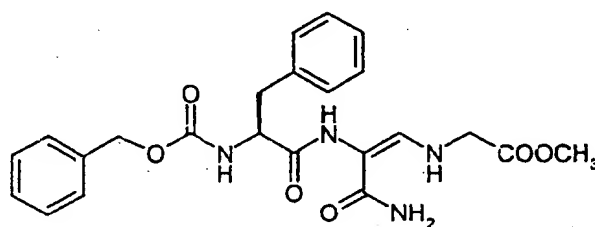
5 Yield; 42%, m.pt. 85-87 °C

NMR(DMSO-d₆): 2.79-3.15(m, 4H), 3.60(s, 3H), 4.16-4.35(m, 2H), 4.96(AB_q, 2H, J= 2.2 and 12.6Hz), 5.42-5.53(m, 1H), 6.15(brs, 2H), 7.08(d, 1H, J= 13.0Hz), 7.20-7.30(m, 15H), 7.79(d, 1H, J= 6.5Hz), 8.71(s, 1H).

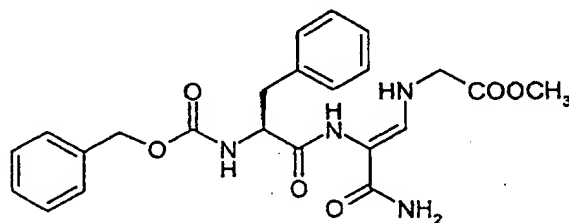
10

Example 31a and 31b

(2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-
acetamido) -3-(1-carbomethoxy-methylamino) -acrylamide (31a)



15 (2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-
acetamido) -3-(1-carbomethoxy-methylamino) -acrylamide (31b)



By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-

2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of glycine

For 31a:

Yield; 36%,

5 m.p. 108-110 °C

NMR(DMSO-d₆): 1.21(t, 3H, J= 7.0Hz), 2.76-3.04(m, 2H),
3.94(d, 2H, J= 6.2Hz), 4.11(q, 2H, J= 7.0Hz), 4.11-4.25(m,
1H), 4.96(s, 2H), 6.02 and 6.53(2brs, 2H), 6.30(d, 1H, J=
12.5Hz), 7.26-7.35(m, 10H), 7.67(d, 1H, J= 6.9Hz), 8.12-
10 8.25(m, 1H), 8.72(s, 1H).

For 31b:

Yield; 41%,

m.p. 145-147 °C

15 NMR(DMSO-d₆): 1.20(t, 3H, J= 7.4Hz), 2.75-3.15(m, 2H),
3.88(d, 2H, J= 5.9Hz), 4.16(q, 2H, J= 7.4Hz), 4.25-4.87(m,
1H), 4.98(s, 2H), 5.22-5.35(m, 1H), 6.20(brs, 1H), 7.05(d,
1H, J= 11.8Hz), 7.19-7.35(m, 10H), 7.75(d, 1H, J= 6.6Hz),
8.70(s, 1H).

20

Biological Example

Testing of inhibitors for inhibition of cathepsin B, L, K
and S.

25

In vitro assay procedure for cathepsin B

The compounds of formula I were tested for inhibition
of cathepsin B using the known method (A.J. Barret et al.,
Biochem. J. 1982, 201, 189-198). To a 170 µl of enzyme-
30 buffer mixture (enzyme: r rat cathepsin B, diluted to
give approximate 10 F units/min, buffer: 56 mM sodium

acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) a 10 μ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μ l of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to
5 initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a
10 linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin L

To a 170 μ l of enzyme-buffer mixture (enzyme: r rat
15 cathepsin L, diluted to give approximate 15 F units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) a 10 μ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μ l of 1 mM substrate (N-CBZ-Phe-
20 Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor
25 concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin K

30 To a 170 μ l of enzyme-buffer mixture (enzyme: r cathepsin K, diluted to give approximate 30 F units/min,

buffer: 100 mM sodium acetate, 5 mM EDTA, 20 mM L-cysteine, 0.01% Brij, pH 5.5) a 10 μ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μ L of 2.7 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin S

To a 170 μ L of enzyme-buffer mixture (enzyme: cathepsin S, diluted to give approximate 30 F units/min, buffer: 100 mM sodium phosphate, 1 mM EDTA, 5 mM DTT, 0.01% Brij, pH 6.5) a 10 μ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μ L of 1.2 mM substrate (N-CBZ-Val-Val-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

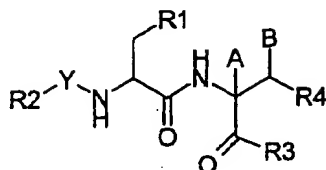
Table 1. In vitro inhibitory activity of compounds on cysteine proteases

Example No.		IC ₅₀ (μM)			
		Cathepsin B	Cathepsin L	Cathepsin K	Cathepsin S
5	1	4.35	0.094	0.011	0.069
	2	1.17	0.072	1.78	0.026
	3	1.42	0.0055	0.23	0.26
	4	2.63	0.015	1.2	0.0087
	5	2.28	0.064	0.0031	0.061
10	6	0.37	0.075	0.003	0.05
	7	1.96	0.23	0.012	0.18
	8	37.1	2.36	0.33	6.9
	9	0.89	0.062	0.014	0.05
	10a	45.21	8.62	1.42	0.013
15	10b	50.51	>64	10.73	>3.2
	11	2.4	0.11	0.014	0.015
	12	1.64	0.076	2.06	0.0035
	13	1.4	0.004	0.4	0.004
	14	0.98	0.004	1.13	0.004
20	15	9.5	0.17	0.018	0.075
	16	0.15	0.015	0.01	0.026
	17	1.9	0.23	0.019	0.18
	18	1.9	0.12	0.0096	0.039
	19				

	19a	>58	>58	>58	1.86
	19b	>58	58	>58	5.23
	20	0.42	0.08	0.004	0.38
	21	8.21	0.21	0.087	0.3
5	22	0.08	0.04	0.04	0.42
	23	0.45	0.089	0.011	0.038
	24	0.082	0.082	0.37	0.0033
	25	0.06	0.18	0.065	0.18
	26	1.47	0.075	0.015	0.99
10	27	32.27	4.24	0.14	52.2
	28	1.96	0.27	0.016	1.3
	29	50.3	2.01	0.34	50.3
	30a	1.83	0.048	0.28	0.57
	30b	1.83	0.035	0.28	1.21
15	31a	0.66	0.017	0.13	0.7
	31b	0.43	0.017	0.09	0.43

CLAIMS

1. A compound of formula (I)



(I)

wherein:

Y represents -C(O)- or -S(O₂)-;

10 R₁ represents a radical of formula R₆-(ALK)_p-(Z)_n-(ALK)_q- wherein Z represents -O- or -S-, ALK represents a divalent C₁-C₃alkyl or halogen-substituted C₁-C₃alkyl radical, p and q are independently 0 or 1, n is 0 or 1 when q is 1 and n is 0 when q is 0, and R₆ is hydrogen or
15 an optionally substituted C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group; or R₁ together with the carbon atom to which it is attached forms a cycloalkyl ring;

20 R₂ represents -OR₅ or -R₅;

R₅ represents a radical of formula R₇-(A)_t- wherein t is 0 or 1; A represents (i) an optionally substituted divalent C₁-C₆alkyl, radical which may be interrupted by one or
25 more non-adjacent -O-, -S- or -NH- linkages, or (ii) a divalent C₂-C₆alkenyl, C₂-C₆alkynyl, cycloalkyl,

cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R_7 represents hydrogen or an optionally substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

5

R_3 represents (i) an optionally substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR_8 or $N(R_8)_2$ or (iii) OR_8 wherein R_8 represents hydrogen or an optionally substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, cycloalkyl, cycloalkenyl or aryl;

10

A and B taken together represent a bond and R_4 represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R_4 each independently represents a hydroxy or substituted hydroxy group;

15

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

20

2. A compound as claimed in claim 1 wherein Y is $-C(O)-$.

3. A compound as claimed in claim 1 or claim 2 wherein R_1 is a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy, methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy-phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl;

25

30

tetrahydroquinolinyl; tetrahydronaphthyl;
aminonaphthyl; or acetamidonaphthyl.

4. A compound as claimed in claim 1 or claim 2 wherein R₁
5 phenyl, isopropyl, cyclohexyl or 3-pyridinyl.
5. A compound as claimed in any of the preceding claims
wherein R₂ is benzyloxy, 3-phenylpropyloxy, 3-
phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-
10 dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl,
methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-
(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-
prop-1-enyl.
- 15 6. A compound as claimed in any of claims 1 to 4 wherein R₃
may be, for example, methyl, ethyl, isopropyl, t-butyl,
cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl,
pyridyl, -NH₂, methylamino, dimethylamino, benzylamino,
piperidino, morpholino, piperazino, N-methylpiperazino,
20 methoxy, ethoxy, t-butyloxy or phenoxy.
7. A compound as claimed in any of the preceding claims
wherein A and B taken together represent a bond, and R₄
is -NH₂, acetylamino, methylamino, dimethylamino,
25 benzylamino, morpholinyl, piperidino, morpholino,
piperazino or N-methylpiperazino, (methoxycarbonyl)-
methylamino, (methoxycarbonyl)-phenethylamino, -OH,
methoxy, allyloxy, benzyloxy.

8. A compound as claimed in any of claims 1 to 6 wherein A represents hydrogen and B and R₄ each independently represents a hydroxygroup.
- 5 9. A compound as claimed in claim 1 which is specifically named and characterised in any of the Examples herein.
10. A pharmaceutical composition containing a compound as claimed in any of the preceding claims and a
10 pharmaceutically acceptable carrier.
11. The use of a compound as claimed in any of claims 1 to 9 in the preparation of a composition for inhibiting cysteine protease activity in the body of a mammal
15 suffering a disease mediated by such activity.
12. A method of treatment of an animal suffering from a disease mediated by cysteine protease activity, which method comprises administering to the mammal a
20 sufficient amount of a compound as claimed in any of claims 1 to 9 to inhibit such activity.
13. The use as claimed in claim 11 or a method as claimed in claim 12 wherein the disease is muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis,
25 neuronal or cardiac ischaemia, allergic immune response, or protozoal or bacterial disease.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/09169 A3

(51) International Patent Classification⁷: C07K 5/062,
5/065, C07D 295/12, A61K 38/05, 31/5375, 31/381, A61P
19/00, 31/00, 35/00, 37/00, 9/00, 25/00

(21) International Application Number: PCT/GB00/02830

(22) International Filing Date: 21 July 2000 (21.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9917909.5 31 July 1999 (31.07.1999) GB

(71) Applicant (for all designated States except US): NAEJA
PHARMACEUTICAL INC [CA/CA]; #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SINGH, Rajeshwar
[CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA). ZHOU, Nian
[CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A
Street, Edmonton, Alberta T6E 5V2 (CA). REDDY,
Andhe, V., N. [CA/CA]; Naeja Pharmaceutical Inc, #2,
4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
THOMAS, George [CA/CA]; Naeja Pharmaceutical Inc,
#2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
DING, Qizhu [CA/CA]; Naeja Pharmaceutical Inc, #2,
4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).
KALETA, Jadwiga [CA/CA]; Naeja Pharmaceutical
Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2

(CA). MICETICH, Ronald, George [CA/CA]; Naeja
Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton,
Alberta T6E 5V2 (CA). WHITTAKER, Mark [GB/GB];
British Biotech Pharmaceuticals Ltd., Watlington Road,
Cowley, Oxford OX4 5LY (GB).

(74) Agents: WALSH, David, Patrick et al.; Appleyard Lees,
15 Clare Road, Halifax HX1 2HY (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

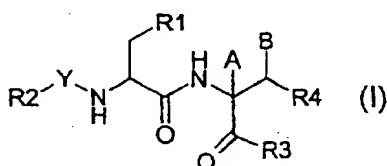
Published:

— with international search report

(88) Date of publication of the international search report:
13 September 2001

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: CYSTEINE PROTEASE INHIBITORS



(57) Abstract: This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins. A compound of formula (I) is described or a pharmaceutically acceptable salt, hydrate or solvate thereof. Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/GB 00/02830

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K5/062 C07K5/065 C07D295/12 A61K38/05 A61K31/5375
A61K31/381 A61P19/00 A61P31/00 A61P35/00 A61P37/00
A61P9/00 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DORAN, JOHN D. ET AL.: "DEACYLATION AND REACYLATION FOR A SERIES OF ACYL CYSTEINE PROTEASES INCLUDING ACYL GROUPS DERIVED FROM NOVEL CHROMOPHORIC SUBSTRATES" BIOCHEMISTRY (1996) 35(38) 12487-12494, XP002163740	
A	WO 96 32408 A (SYNPHAR LAB INC) 17 October 1996 (1996-10-17) cited in the application	
A	WO 98 12176 A (SYNPHAR LAB INC) 26 March 1998 (1998-03-26) cited in the application	
	--- -/-- ---	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *G* document member of the same patent family

Date of the actual completion of the international search

23 March 2001

Date of mailing of the international search report

05/04/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/GB 00/02830

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BAGGIO RICKY ET AL: "From poor substrates to good inhibitors: Design of inhibitors for serine and thiol proteases." BIOCHEMISTRY, vol. 35, no. 11, 1996, pages 3351-3353, XP002163741 ISSN: 0006-2960</p> <p>-----</p>	
A	<p>DUFFY K J ET AL: "Design and synthesis of diaminopyrrolidinone inhibitors of human osteoclast cathepsin K" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,GB,OXFORD, vol. 9, no. 14, 19 July 1999 (1999-07-19), pages 1907-1910, XP004171607 ISSN: 0960-894X</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02830

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9632408 A	17-10-1996	AT 197591 T	15-12-2000
		AU 4951896 A	30-10-1996
		CA 2212356 A	17-10-1996
		DE 69610978 D	21-12-2000
		EP 0817795 A	14-01-1998
		JP 11503728 T	30-03-1999
		US 5986108 A	16-11-1999
WO 9812176 A	26-03-1998	AU 4133697 A	14-04-1998
		EP 0950046 A	20-10-1999
		US 5959123 A	28-09-1999